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RESEARCH ARTICLE

PEANUT OIL BASED W1/O/W2MULTIPLE EMULSIONS FOR ORAL ADMINISTRATION OF INSULIN

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ABSTRACT

In this study it was performed multiple emulsions of water in oil in water (W1/O/W2) containing insulin. The aim was to isolate the insulin molecule in emulsions of this type for optimal protection against digestive degradation. These emulsions were made in two steps, a first step of formulating a water-in-oil emulsion wherein the oil phase consists of peanut oil, not frequently used and tested in these types of emulsions, and a second one of multiple emulsion formulation. In the methodology, parameters such as the hydrophilic / lipophilic balance, the type of surfactant, the amount of thickening in the internal and / or external phase, the proportions of different fractions introduced, the speed and stirring time, were varied. The control of all these parameters allowed to produce emulsions that have superior long-term stability to one month. Stability tests in varying pH environments simulating those in the stomach and small intestine have also given satisfactory results. It plan later to continue the stability studies of this emulsions by further in vitro studies, but also by in vivo studies in diabetic rats.

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INTRODUCTION

Nowadays, emulsions are increasingly used in the field of research particularly in the pharmaceutical field where many applications have emerged. The emulsions are used for delivery of drug such as anticancer, anti-inflammatories, antibiotics (Omotosho *et al*., 1990) (Cournarie *et al*., 2004) (D.J. Mc Clements *et al*., 2007). For protecting molecules which are introduced into the internal phase; but also for the administration of two different solubility molecules, particular emulsionssaid multiple emulsions are used (Chao-Cheng Chen *et al*., 1999) (Hino *et al*., 2001). By this technique, it is possible to mask an unpleasant taste; to protect an active molecule against oxidation or enzymatic degradation including digestive enzymes (Silva-Cunha *et al*., 1997). Many factors contribute to the stability of emulsions (Jie Li *et al*., 2015). The objective in the emulsions is to reduce the interfacial tension between the two liquids but also maintain sufficient pressure inside the droplets formed for improved stability (Kanouni *et al*., 2002). We made multiple emulsions of water-in-oil-in-water (W1 / O / W2) containing insulin.

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The process of emulsification was conducted in two stages, a first step of formulation of water-in-oil emulsion and a second step of formulating multiple emulsions. In the formulation of these emulsions, the following parameters have been modified:

- The hydrophilic / lipophilic balance,
- The type of surfactant,
- The quantities of thickening agents in the internal and / or external phase,
- The proportions of the various fractions introduced,
- The stirring speed and the time.

MATERIALS AND METHODS

Reagents and equipment

The active ingredient used was bovine insulin powder from Sigma Aldrich laboratories. As emulsifiers we used the sorbitanmonooleate or Span 80®, sorbitantrioleateor Span 85®, sorbitanmonooleate, polyethylene glycol or Tween 80 ®, polyethylene glycol sorbitantrioleate Span 85® from Sigma Aldrich laboratories and Montane 481 VG ®, from SEPPIC, consisting of a mixture Sorbitanoleate, beeswax, castor oil with

saturated fatty acids, and stearic acid. The lipophilic phase consists of peanut oil and the hydrophilic phase a phosphate buffered saline (PBS) containing hydroxyethylcellulose or Natrosol ® from Aqualon laboratories. As devices we used magnetic stirrers Heidolph-type model MR3001K, optical microscope Axio Zeiss imager A1 coupled to a computer containing the Axio Vision software rel. Version 4.5, a precision balance type Ohaus explore, and a tensiometer Dognon-Abribat PROLABO model.

Methods

Determination of HLB required for the W1 / O emulsion

For the inverse emulsions, required HLB should be between 3 and 6. In the formulations it has been carried to a variation of the hydrophilic lipophilic balance using emulsifying agents such as sorbitantrioleate or Span 85 ® (HLB 1.8) and polyethylene glycol sorbitantrioleate or tween 85 ® (HLB 11). The different HLB given by the couple Tween 85 / Span 85 were obtained using the following equation (ICI Americas Inc. Wilmington 1980):

$$\%\ \text{Tween 85} = \frac{100 \quad (X - HLB_{\text{span 85}})}{HLB_{\text{Tween 85}} - HLB_{\text{Span 85}}}$$

$$\%\ \text{Span 85} = 100 - \%\ \text{Tween 85}$$

X is the value of the desired HLB.

In the Table 1 are given the different proportions.

Table 1. Proportions of emulsifier Span 85 ®/ Tween 85® for HLB ranging from 2 to 6

Tween 85 ®	Span 85 ®	HLB
2%	98%	2
13%	87%	3
24%	76%	4
35%	65%	5
46%	54%	6

With regard to the Montane 481 VG ®, it is composed of a mixture for which the HLB values (4.5)is anHLB within the range permitting achievement of inverse emulsions in which it was carried out a variation of proportions used. After weighing the quantities required, we mixed the montane with peanut oil at 80 ° C then allowed to cool at 40 °C before proceeding to the emulsion.

Determination of HLB required for W1/O/W2 emulsion

The same process as mentioned above was used for this step. The emulsifiers which have been used are couples span 80 ® / tween 80 ® whose HLB ranges from 4.3 to 15. The proportions of emulsifiers for different HLB required are summarized in the Table 2.

Determination of emulsifying agent proportions

For couples span® / tween® and for the montane 481 VG® we used different proportions summarized in Table 3.

Table 2. Proportions of emulsifier Span 80 ®/ Tween 80® for HLB ranging from 7 to 14

Tween 80 ®	Span 80 ®	HLB
25,23%	74,77%	7
34,58%	65,42%	8
53,27%	46,73%	10
71,96%	28,04%	12
90,65%	9,35%	14

Table 3. Proportions of emulsifiers used

Tube number	(%) of emulsifier
1	5
2	10
3	15
4	20

Study of the stability of emulsions in varying pH media

This study was performed in citrate buffer environments at 37° C with constant stirring. To do this, we used dialyzing membrane for diffusion of the buffer solution inside, this in order to be able to pass the same sample in various buffer media whose pH are equal to, 2 corresponding to the pH of the stomach and two others having a pH of 6 and 7.3 corresponding to that of the small intestine. These dialyzing type membranes have the advantage to let through only small molecules corresponding to the electrolytes present in the buffer solution.

RESULTS AND DISCUSSION

To determine the HLB required for the W1/O emulsion, the method is based on the classification developed by Griffin (Griffin 1949). The basic hypothesis states that for a given fatty phase, there is an optimum HLB for obtaining maximum stability. We have had relatively satisfactory results with the span 85 ®/ tween 85 ® for wich we found HLB equal to 3 as shown in Table 4.

Table 4. Stability of emulsions formed with span 85 ®/ tween 85 ® couple at various HLB

Samplenumber	HLB	Results (number of phases at j 5)
1	2	2
2	3	1 (±)
3	4	2
4	5	2
5	6	3

Indeed for such couples span 85 ®/ tween 85 ®, the stability does not exceed 5 days. We have not done any studies on the speed of agitation or stirring time, however, we estimate that the HLB about 3 could give satisfactory results.

We dwell on the montane 481 VG specially used for inverse emulsions in composition (SEPPIC 2015). For the proportions used, we noticed that the emulsions have started giving creams from 10%, which justified the restriction of our study in proportions of between 0 and 8%. For these concentrations of montane 481 VG ® we measured the surface tension of which the results are shown in Figure 1.

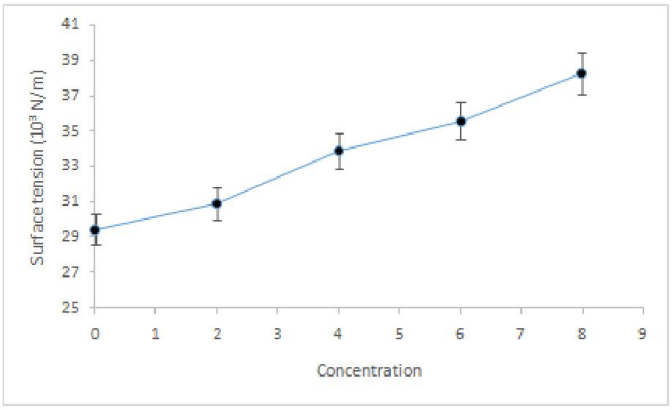

Figure 1. Changes in surface tension as a function of the montane 481VG ® concentration

These results show that the montane 481VG® increases the surface tension instead of diminishing it. This may be justified to the extent that we have emulsions in which it is the densest phase to be introduced in the less dense phase hence the necessity to increase the density of the latter to prevent sedimentation of the internal phase through a gravity phenomenon (Handbook 1967).

With regard to stability, the results show that the emulsions using the montane 481VG® with an HLB equal to 4.5 give satisfactory results except for the first tube in which the emulsions are not stable as shown in Table 5.

Table 5. Results of montane 481 VG ® based emulsions

N° Sample	Composition	Results (number of phases j 5)	Stability
1	5%	1 (fluid)	+/-
2	6%	1 (fluid)	++
3	7%	1 (fluid)	++
4	8%	1 (cream)	++

For multiple emulsions, the results show that couples span 80 ® / tween 80 ® which give an HLB equal to 8, allow stable emulsions in proportions of 10%. In both the inner and outer phase we found also that the proportion of 1% of Natrosol® give stable emulsion.

During the formulation we found a stirring speed of1250 rpm for 10 min for W1/O emulsion and 700 rpm for 5 min during the formulation of the multiple emulsion. The study of the stability of the emulsions, after contacting with different pH buffer media for a duration of 1 hour, gave the results shown in Figure 2. These results show that the emulsions remained intact during their stay at these environments which simulate the pH conditions of the stomach and intestine.

Conclusion

Insulin is a protein, the main obstacle in its oral administration is its degradation by digestive enzymes such as pepsin but also the pancreatic proteases such as trypsin and chymotrypsin. The aim of our study was therefore to isolate the insulin molecule in multiple emulsions for optimal protection.

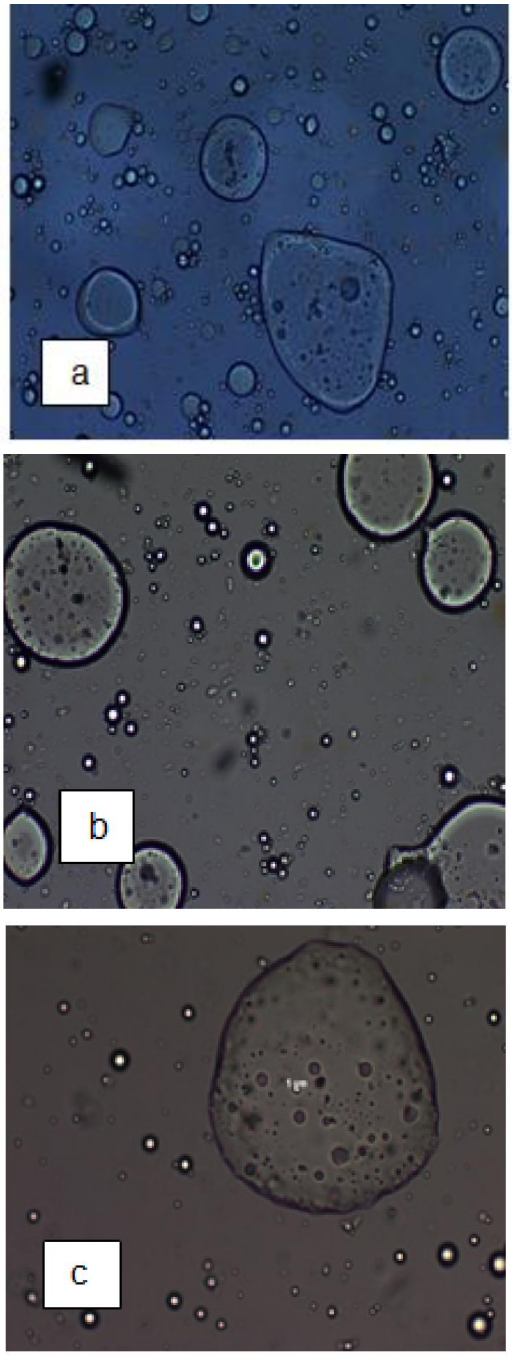

Figure 2. Microscopic view of emulsions after contact at 37°C pH 2.1 one hour (a),pH 6 one hour (b) and pH 7.3 at the same conditions (c) (X100)

So we had to vary parameters such as the hydrophilic / lipophilic balance, the type of surfactant, the amount of thickening agents in the internal phase and / or external, the proportions of different fractions introduced, the speed and stirring time. The control of all these parameters allowed us to produce emulsions that have had lasting stability greater than one month. Stability tests in varying pH environments simulating those in the stomach and intestine have also given satisfactory results. We plan later to continue the studies of stability of our emulsions through further in vitro studies, but also by in vivo studies in diabetic rats.

REFERENCES

Chao-Cheng Chen, Yen-Ying Tu, and Hung-Min Chang. 1999. "Efficiency and Protective Effect of Encapsulation of Milk Immunoglobulin G in Multiple Emulsion J. *Agric. Food Chem.,* 47 (2), pp 407–410.

Cournarie F, Rosilio V, Cheron M, Vauthier C, Lacour B, Grossiord J L, Seiller M. 2004. Improved formulation of W/O/W multiple emulsion for insulin encapsulation. Influence of the chemical structure of insulin. Colloid Polym Sci 282: p 562-568.

Griffin, W. C. 1949. Classification of surface-active agents by "HLB". *J. Soc. Cos. Chem.,* 1: p 311-326.

Hino, T, Shimabayashi, S, Tanaka, M, Nakano, M, Okochi, H. 2001. Improvement of encapsulation efficiency of water-in-oil-in water emulsion with hypertonic inner aqueous phase *Journal of Microencapsulation: Micro and Nano Carriers,* Volume 18, Issue 1.

ICI Americas Inc. Wilmington, 1980. Delaware 19897. The HLB system, a time saving guide to emulsifier selection. Chemmunique, publication of ICI Americas Inc.

Jie Li, Lin Su, Jing Li, Mei-Fang Liu, Su-Fen Chen, Bo Li, Zhan-Wen Zhanga and Yi-Yang Liu, 2015. Influence of sucrose on the stability of W1/O/W2 double emulsion droplets, RSC Adv., 5, 83089-83095.

Kanouni M, Rosano H L, Naouli N. 2002. W1/O/W2: role of the interfacial films on the stability of the system. Advances in Colloid and Interface Science. 99: p 229–254.

Lange's Handbook of Chemistry 1967, 10th ed. pp 1661–1665."

Mc Clements D.J., Decker E.A. and Weiss J.. 2007. Emulsion-Based Delivery Systems for Lipophilic Bioactive Components. *Journal of Food Science*, Vol 72, 8: p R109-R124.

Omotosho J. A., Florence A. T. and Whateley T. L. 1990. Absorption and lymphatic uptake of 5-fluorouracil in the rat following oral administration of W/O/W multiple emulsions. *Int. J. Pharm. 61,* p 51-56.

SEPPIC. http://www.seppic.com/cosmetique/emulsionnant-eau-huile/origine-vegetale-montane-@/view-383-seproduit.html;jsessionid=hPCICJQjvUvNGOLvKfO8Cg__?lang=fr. 2015. (accessed 04 23, 2015).

Silva-Cunha, A., Grossiord, J.L., Puissieux, F. and Seiller, M. 1997. W/O/W multiple emulsions of insulin containing a proteas inhibitor and a absorbtion enhancer: preperation, characterization, and determination of stability towards proteases in vitro. *International journal of pharmaceutics*, 158: p 79-89.

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